A Clinical Approach to Antioxidant Therapy: Hypertonic Fluid Resuscitation Trial

Final Report

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Abstract

It is well established that severe trauma with hemorrhagic shock produces significant immunomoduation, including enhanced PMN activation, adherence and emigration into tissues, along with the induction of inflammatory cytokine cascades, which collectively contribute to the systemic inflammatory response syndrome (SIRS), frequently culminating in acute respiratory distress syndrome (ARDS) and multiple organ dysfunction syndrome (MODS). The hemodynamic and physiological benefits of hypertonic saline / dextran solutions (HS/D) have been evaluated extensively in animal and human research, and efficacy has been suggested for the treatment of hemorrhagic shock. Work from our research group and others has substantiated that in comparison to large volume crystalloid resuscitation, small volume resuscitation with HS/D has the remarkable capacity to reduce potentially deleterious immunologic complications. However, human clinical studies evaluating the cellular and molecular immune consequences of small-volume HSD resuscitation are lacking; also there is need to investigate modified fluid formulations incorporating novel antiinflammatory agents such as the antioxidant N-acetylcysteine.

Résumé

Il est bien établi qu'un traumatisme grave s'accompagnant d'un chox hémorragique produit un effet immunomodulateur important, notamment une activation accrue des PMN, l'adhérence et la migration dans les tissus ainsi que l'induction de cascades cytokiniques inflammatoires, qui contribuent collectivement au syndrome de réponse inflammatoire systémique (SRIS), aboutissant souvent au syndrome de détresse respiratoire aiguë (SDRA) et au syndrome de défaillance multiorganique (SDMO). Les bienfaits hémodynamiques et physiologiques des solutions hypertoniques salines/dextran (HSD) ont été évalués amplement dans des recherches chez l'animal et l'homme, et leur efficacité a été évoquée pour le traitement du choc hémorragique. Les travaux de notre groupe de recherche et d'autres équipes ont confirmé que, comparée à une réanimation au moyen d'un fort volume de cristalloïdes, la réanimation au moyen d'un faible volume de solution HSD a la remarquable faculté de réduire les complications immunologiques potentiellement délétères. Cependant, il

faudrait des études cliniques évaluant chez l'homme les conséquences immunes cellulaires et moléculaires de la réanimation au moyen d'un faible volume de solution HSD; de plus, il faudrait étudier des formules liquides modifiées comportant des agents anti-inflammatoires nouveaux, tels que la N-acétylcystéine antioxydante.

Executive summary

At present, there is no clear consensus regarding the optimal treatment of hemorrhagic shock and continued controversy exists regarding the most appropriate fluid strategy for combat casualties. A safe and effective fluid that can be used in smaller amounts to resuscitate injured soldiers would enhance mobility in the field and improve the treatment of casualties in situations where supplies are limited. A promising alternative treatment involves small-volume infusion of hypertonic saline (7.5% NaCl); infusion of 250 mL (~1 cup) of hypertonic saline expands plasma volume to the same extent as >10 times higher volume of isotonic saline. This small amount of hypertonic saline immediately corrects blood pressure and other hemodynamic parameters of patients in hemorrhagic shock. The amount is easily carried by corpsmen, can be infused within minutes and, because of its long-lasting effects, reduces the need for other fluids. Moreover, our studies have demonstrated that hypertonic saline exerts beneficial anti-inflammatory effects, which can down-regulate neutrophil activation and reduce post-traumatic multiple organ dysfunction.

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À l'heure actuelle, il n'existe pas de véritable consensus entourant le traitement optimal du choc hémorragique, et la controverse persiste en ce qui concerne la stratégie qui convient le mieux pour le remplacement des liquides chez les soldats blessés au combat. Un liquide sûr et efficace qui peut être utilisé en plus petites quantités pour ranimer des soldats blessés permettrait d'assurer une plus grande mobilité sur le terrain et d'améliorer le traitement des victimes dans les situations où les fournitures sont limitées. Un traitement de remplacement prometteur consiste à perfuser un faible volume d'une solution saline hypertonique (NaCl à 7,5 %); 250 ml (~1 tasse) de solution saline hypertonique accroît le volume plasmatique autant qu'une solution isotonique à un volume plus de dix fois plus élevé. Cette petite quantité de solution saline hypertonique corrige immédiatement la pression artérielle et les autres paramètres hémodynamiques chez les patients en état de choc hémorragique. Cette quantité peut facilement être transportée par le personnel, elle peut être perfusée en quelques minutes et, compte tenu de ses effets durables, elle réduit le besoin d'autres liquides. De plus, nos études ont démontré que la solution saline hypertonique a des effets anti-inflammatoires bénéfiques qui peuvent atténuer l'activation des neutrophiles et réduire la défaillance multiorganique post-traumatique.

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1. Introduction

1.1 Background

The epidemiology of morbidity and mortality associated with both military and civilian trauma has evolved significantly over the past several decades. In the civilian setting, rapid evacuation and transfer of traumatized patients to specialized centres with en route aggressive fluid resuscitation and airway support has reduced acute mortalities at the injury scene.[1] In addition, improved training of surgical teams has facilitated triage and care, resulting in improved survivorship in the early post trauma period.[2] Yet, a significant number of survivors still go on to develop Multiple Organ Dysfunction Syndrome (MODS) and ultimately die.[3,4]

On the battlefield, historically, about 20% of all injured soldiers die, with 90% of the deaths occurring before reaching a field hospital.[5,6] Recent statistics show that 50% of deaths in potentially salvageable combat casualties are due to acute hemorrhage, making it the leading cause of death on the battlefield.[7,8] An estimated 80% of combat casualties require intravenous fluids and prompt control of bleeding and resuscitation in the field can reduce mortality by as much as 20%.[9] Nevertheless, there is no clear consensus regarding the optimal treatment of hemorrhagic shock and continued controversy exists regarding the most appropriate front-line fluid resuscitation strategy for treating hypotensive combat casualties.[10-13] Moreover, what may be ideal doctrine for the treatment of civilian trauma casualties in an urban center, with rapid evacuation, relatively short transport times and access to unlimited medical resources, may be inappropriate for the medic in an austere far-forward combat setting.[14] Thus, it is important to establish a standards of care that are practical and appropriate for modern military environments, which often entail: tactical considerations, direct hostile fire, mass casualties, limited forward surgical care and delayed evacuation.[9]

1.1.1 Current Fluid Resuscitation Standard of Care

By virtue of clinical experience, low cost, and wide availability, large volume infusion of isotonic crystalloid (i.e., 0.9% NaCl) solutions (e.g., lactated Ringer's [LR] and normal saline [NS]), have remained the standard of care for almost a century for the resuscitation of hypovolemic trauma patients, in both military and civilian pre-hospital settings.[15,16] Current ATLS guidelines[17] recommend prompt restoration of tissue perfusion using isotonic crystalloids,

administered in a volume approximately three-times that of estimated blood loss (up to three 20 mL/kg aliquots). Although conventional wisdom has been to provide such aggressive blood pressure support through liberal use of isotonic solutions, there is ongoing debate whether or not early aggressive fluid replacement therapy may be detrimental.[18]

While restoration of intravascular volume and pressure is intuitively logical, attempts to achieve normal arterial pressure using large-volume infusions increases the risk of blood loss before hemostasis or by re-bleeding, thereby potentially increasing mortality. Indeed, evidence from experimental animal studies suggests that small-volume hypotensive resuscitation results in improved long-term survival.[19-21] To date, the only published study in humans has demonstrated a significant reduction in mortality when fluid was restricted in the prehospital period.[22] However, this study has been criticized for a number of methodological weaknesses (i.e., patient heterogeneity, retrospective stratification of patients, large differences in fluid volume infused).[1] In addition, this study was conducted in a large urban center, with relatively short transport times from the scene to the operating room and was restricted to patients with penetrating torso trauma and it remains unclear whether victims of multi-system blunt trauma with delayed transport times would respond similarly to limited resuscitation.[23]

1.1.2 Adverse Effects of Aggressive Fluid Resuscitation

Large volume fluid resuscitation with crystalloids can provoke a number of adverse reactions. Chief among these is the capacity to amplify harmful immuno-inflammatory responses, including neutrophil activation, initiating the development of multiple organ dysfunction.[24] The pathologic changes in organs affected by MODS are characterized by the presence of diffuse inflammation with leukocyte infiltration and interstitial edema the most common findings at autopsy.[25] In fact, many of the complications following the resuscitation of hemorrhagic shock may be related to alterations in host immunity. In particular, the polymorphonuclear neutrophil (PMN), one of the principal host immune effector cells, has been implicated as a key mediator of tissue damage leading to organ injury and MODS after hemorrhagic shock resuscitation and other systemic inflammatory conditions.[26] Although the PMN is essential in protecting the host from traumatic and infectious insults, it may also turn its potent defenses inappropriately against the host, contributing to the severity of injury.[27] Under physiologic conditions, passage of PMNs from the microcirculation into tissue involves an ordered sequence of steps characterized by PMN rolling, adhesion to endothelium, and transmigration.[28] PMN extravasation into inflamed or injured areas involves a complex interaction of leukocytes with endothelial cells via regulated

expression of surface adhesion molecules. The initial attachment of PMNs to endothelium is mediated by L-selectin (CD62L). L-Selectin is constitutively expressed by PMNs and is released from neutrophils by a proteolytic cleavage within minutes after activation with a concomitant upregulation of Mac-1 (CD11b/CD18). The CD18 integrins, Mac-1 and LFA-1 (CD11a/ CD18), are largely responsible for subsequent tightening of the adhesion and transendothelial migration of neutrophils via interactions with their endothelial counter-receptors, ICAM-1 and ICAM-2.[29] The inappropriate upregulation of these PMN-endothelial cell interactions in the ischemia/reperfusion injury of hemorrhagic shock resuscitation is believed to be an important step in the host's progression to systemic inflammation and subsequent remote organ injury.[30] After resuscitated hemorrhagic shock, aberrant and unbridled activation of sequestered PMNs unleash a cytotoxic arsenal of oxygen radicals and proteinases, causing injury to endothelium and resulting in vascular leakage, tissue edema, and eventually organ damage.

The lungs are an important target organ of the systemic inflammatory response observed after major injury[31]; as reflected in the high proportion of trauma patients that subsequently develop Adult Respiratory Distress Syndrome (ARDS).[32] ARDS is characterized by the clinical picture of hypoxemia, reduced lung compliance and diffuse alveolar infiltrates. Injury to the capillary endothelium and alveolar epithelium results in the loss of the barrier function of the alveolar-capillary membrane and is responsible for leakage of fluid and inflammatory cells into the interstitium and the alveolar space.[33] PMNs traversing the capillary bed in the lung are trapped due to decreased deformability and specific adherence to the endothelium.[34] In humans, the extent of PMN influx and the presence of PMN products in the alveolar lavage fluid have been correlated with the severity of the lung injury.[35]

The development of ARDS in traumatized patients is a significant contributor to their morbidity and mortality. Hemorrhagic shock is believed to contribute to the pathogenesis of ARDS by rendering the patient more susceptible to a second, seemingly trivial, inflammatory stimulus, the so-called "two-hit" model.[36] In essence, the resuscitated shock phase acts to prime the immune system for an over-exuberant response to a second, often minor, inflammatory insult. Since PMN accumulation in the lung is felt to contribute to the pathogenesis of tissue injury, studies have suggested that ischemia-reperfusion can mediate this effect by priming circulating PMNs for increased superoxide production, with the result that cytotoxicity is enhanced once the PMNs are sequestered in the lung.[33]

1.1.3 Benifits of Hypertonic Saline Resuscitation

The benefits of small-volume (4 mL/kg) hypertonic resuscitation as the initial therapy for severe hypovolemia and shock were advocated almost two decades ago.[37] Following the demonstration by Holcroft and others that hypertonic (7.5% saline) / hyperoncotic (6% Dextran-70) solutions (HS/D) could restore blood pressure and cardiac output at a volume dose of about 1/10 of conventional crystalloid solutions, several clinical investigations have been conducted using HS/D for initial resuscitation from hemorrhagic shock.[38,39] Infusion of 250 mL of HS/D creates a potent transcapillary osmotic gradient causing intravascular movement of water from the interstitium, endothelial cells and red blood cells, rapidly expanding plasma volume, improving blood viscosity through hemodilution and reduction in erythrocytes size.[40,41] This quickly restores mean arterial pressure, peripheral tissue perfusion, myocardial contractility, and oxygen consumption, via vasodilatation of precapillary resistance vessels, direct myocardial stimulation, and increases in cardiac pre-load.[42] Collectively, these processes could reduce third-space fluid sequestration in the lungs of patients with ARDS.[43]

In addition to the intravascular volume expansion, HS/D infusion has favorable immunomodulatory effects.[24,44,45] Although the clinical trials that tested hypertonic solutions in civilian trauma patients have individually failed to provide convincing evidence of efficacy,[46-49] a recent meta-analysis (of 8 trials) has suggested a survival advantage of 37.9% versus 26.9% for HS/D over standard of care.[39] At least one multicentric randomized control trial has demonstrated fewer post-resuscitation complications, such as ARDS, renal failure, and coagulopathies, with the use of HS/D.[47] Substantial evidence, from cellular and animal research, reveal that HS/D resuscitation of hemorrhagic shock alters PMN structure and function.[29,50-58] For example, exposure of human PMNs to hypertonic media *in vitro* reduces their cytotoxicity, cellular activation, and the expression of surface adhesion molecules. *In vivo* studies show that both PMN and endothelial adhesion molecule expression is reduced in HS/D-resuscitated animals as compared to those receiving LR. These alterations in adhesion molecules suggest that HS/D may impart functional changes in PMNs and/or endothelial cells. More importantly, animal models of hemorrhagic shock resuscitated with HTS have shown reductions in lung injury, diminished lung PMN infiltration, and pulmonary myeloperoxidase as well as decreases in mortality. Shrinkage of PMNs and cytoskeletal alterations through differential phosphorylation of membrane kinases are believed to be some of the possible mechanisms by which HS/D may promote these changes in PMN function and structure.[45] However, to date, these promising immunomodulatory effects have not been confirmed in well-controlled clinical human trials of HTS.

1.2 Study Rationale

The use of hypertonic fluids for the treatment of hemorrhagic hypotension has the potential to offer superior hemodynamic and immunologic effects, which can reduce the risk of post-traumatic multiple organ dysfunction. In addition to these clinical advantages, adoption of hypertonic fluids for combat resuscitation could provide a substantial logistical advantage over conventional fluid therapy for the rapidly mobile fighting forces of the future by dramatically decreasing the volume of fluid required for resuscitation in the far-forward combat arena.

1.3 Statement of Objectives

Based on these considerations, the purpose of the present study was to evaluate the role of small-volume hypertonic fluid reuscitation strategies on patient outcome, cellular and molecular mechanisms of immuno-inflammatory activation and the prevention and management of acute lung injury in the clinical trauma setting.

1.4 Work Statements

1. Design and conduct a clinical study of the efficacy and outcome of applying a small-volume resuscitation strategy, using hypertonic saline/dextran (HS/D), with or without the antioxidant N-acetylcysteine, in hypotensive patients. The study was conducted with written approval by the hospital human ethics committee. **(Aim 1)**
2. Conduct laboratory studies to determine the feasibility of liposomal antioxidants administered via the intravenous route for the resuscitation of hemorrhagic shock, and determine the underlying mechanisms of associated organ injury and its reversal by the treatment. **(Aim 2)**
3. Conduct toxicological evaluation of liposomal NAC formulation in animals and submit toxicological data to the Health Protection Branch of Health and Welfare Canada for preliminary approval for a preliminary experimental human study. The animal toxicological evaluation of liposomal NAC will be divided into four parts: a) potential pharmacologic interactions among different components of the liposomal NAC formulation; b) toxicokinetic profile of the formulation; c) acute toxicological study; and d) sub-chronic toxicological study. **(Aim 3)**

2. Experimental Section

2.1 Hypothesis

It is hypothesized that the use of hypertonic (7.5% saline) / hyperoncotic (6% Dextran-70) solutions (HS/D) in the early management of trauma patients might exert beneficial immunomodulatory / anti-inflammatory effects, which might lessen the probability of lung injury. Specifically, HS/D will reduce cytokine-induced PMN activation, as measured by changes in cell-surface and circulating cellular adhesion molecule levels and it can be further hypothesized that the priming event of resuscitated shock might occur through inhibitory influences on the inflammatory cell signalling in the lung, an effect which might be sensitive to antioxidants.

2.2 Materials and Methods

2.2.1 Study Design and Patient Selection

A total of 94 eligible blunt and penetrating trauma patients were enrolled into this prospective, double-blind controlled trial, over a 14-month period (between May 2001 to July 2002), of whom 27 were randomized (using a random numbers table) to either normal saline control (NS; n = 14) or hypertonic saline–dextran (HSD; n = 13) resuscitation treatment groups (**Figure 1**). Patients were included in the study if they had at least one recorded episode of hypotension (systolic blood pressure ≤ 90 mm Hg) from hemorrhagic shock; they were 18 years of age or older; they had sustained trauma caused by blunt or penetrating injury within the last 12-h; and, as per the investigator's judgment, they were expected to survive for at least 24 hours. The exclusion criteria were pregnancy, associated illness, cardiac arrest or imminent death. Institutional review board approval was obtained at each of the participating sites. Delayed / waived consent was obtained from all patients or their next of kin.

Upon arrival, trauma patients in hemorrhagic shock randomly received a 250 mL bolus infusion of either hypertonic saline in dextran (HSD; 7.5% NaCl / 6% dextran 70) or normal saline (NS) from unidentified bags. Subsequent resuscitation adhered to ATLS® guidelines. Injury and disease severity were scored according to Severity of Injury Score (SIS) and Acute Physiological and Chronic Health Evaluation (APACHE) II scoring systems.

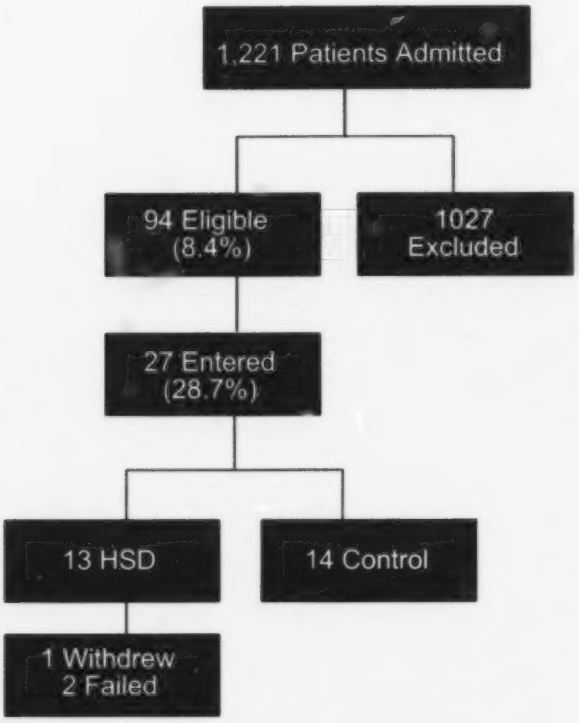

Figure 1. Patient Selection

2.2.2 Blood Sample Collection and Incubation

Peripheral venous blood samples were obtained serially from trauma patients at five time points: pre-resuscitation (baseline; 0-h), 1, 3, 6, and 24-h after fluid resuscitation (**Figure 2**). Specimens for hematological and flow cytometric cellular adhesion molecule analyses were collected into 3 mL sterile glass vacutainers (Becton Dickinson, Franklin Lakes, NJ), containing EDTA and sodium heparin, respectively. After sampling, 1-mL aliquots of heparinized whole blood were mixed 1:1 with RPMI 1640 culture medium and either stimulated with 1 μg/mL lipopolysaccharide (LPS; *Escherichia coli* 055:B5; Sigma, St. Louis, MO) or RPMI only (unstimulated). Stimulated and unstimulated samples were incubated for 22-h at 37°C in a 5% CO_2 humidified atmosphere. Five mL blood samples for measurement of soluble L-selectin (sCD62L) were drawn into non-additive vacutainers and the serum was separated at 1000 x *g* for 15-min, aliquoted and immediately frozen at –80°C until the time of assay.

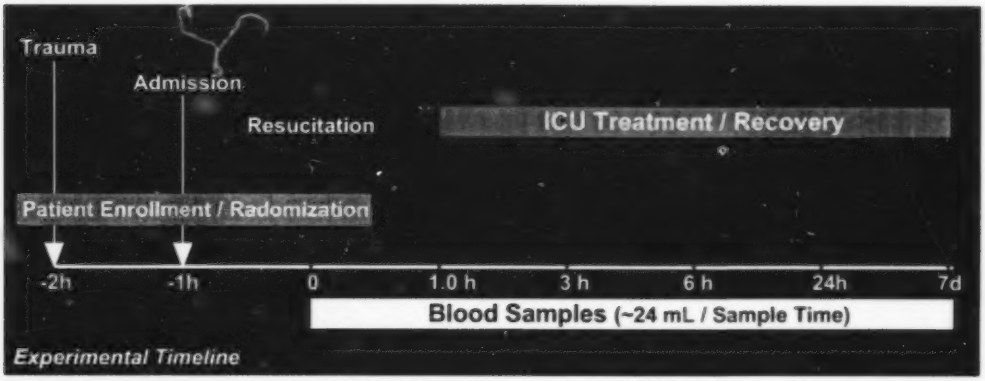

Figure 2. Experimental Timeline

2.2.3 Whole Blood Assay of Cellular Adhesion Molecule Expression

Four-colour flow cytometric analysis of cellular adhesion molecule expression, by circulating neutrophils and monocytes, was performed on a Becton Dickinson FACScalibur flow cytometer (BD Biosciences, San Jose, CA) using a standardized whole blood staining technique. Briefly, 100-µL aliquots of heparinized blood were incubated, at room temperature for 30 min in the dark, with saturating concentrations of monoclonal antibodies (mAb) directly conjugated with fluorescein isothiocyanate (FITC), phycoerythrin (PE), allophycocyanin (APC) and Peridinin Chlorophyll Protein (PerCP), directed towards CD11b (CD18 β_2 subunit), CD62L (L-selectin), CD45, and CD14, respectively. The respective isotype-matched negative control mAbs were added simultaneously to separate tubes at identical concentrations for all samples to detect non-specific binding. Erythrocytes were lysed for 10 min using 2 mL of 1X FACS™ Lysing Solution. The tubes were then centrifuged for 5 min at 300 x *g*. After washing in 2 mL of PBS containing 0.1% sodium azide the cells were resuspended and stored in 300 µL of PBS containing 1% paraformaldehyde until acquisition within 24-h. A minimum of 30,000 events for each antibody combination was acquired and saved for later analysis using CellQuest™ Pro software. The results are expressed as the proportion of postive cells and relative fluorescence intensity for a particular marker. The percentage of granulocytes and monocytes was defined by setting region gates for each leukocyte population based on two-dimensional dot-plot graphs of CD45–PerCP / CD14–APC fluorescence characteristics. For analysis of cellular adhesion molecule expression by neutrophils and monocytes, the intensity of receptor expression (i.e., density per cell) was

summarized from fluorescence histogram data as mean fluorescence intensity (MFI) ± confidence intervals (CI), in arbitrary units (au) on a $\log_{10}$ scale (from 1 to 10,000). For each population, a class-matched isotype-control mAb was used to define the placement of the negative marker; negative regions typically included ≥98% of the control events. Fluorescence intensity was standardized for each cytometer run using CaliBRITE™ beads and AutoCOMP™ software.

2.2.4 Circulating Soluble L-Selectin (sCD62L) Concentration

Serum concentrations of soluble L-selectin (sCD62L) were measured with a sandwich enzyme-linked immunoabsorbent assay (ELISA) kit (Bender MED-Systems, Vienna, Austria). The assay was performed according to the manufacturer's instructions, and all samples were analyzed at a 1:200 dilution resulting in concentrations within the range of the standard curve. Optical density, with wavelength correction, was read using an automated microplate photometer (EL340, BIO–TEK Instruments, Winooski, VT). The detection limit of human sL-selectin is 0.3 ng/mL. All results from the ELISA measurements represent the means from duplicate measurements; and the differences between duplicates were generally ≤10%.

2.3 Data Analysis

Immunological data are expressed graphically as the mean and the 95% confidence intervals and as maximal percental changes for description in the text. To examine differences between resuscitation treatment groups (NS vs. HSD) and the effects of time on immune parameters, a two-way repeated measures analysis of variance (ANOVA) was performed. When a significant *F*-ratio was obtained, pair-wise post-hoc comparisons were performed to isolate differences among treatment means using a Huynh-Feldt correction for multiple comparisons. For all comparisons, a probability of less than .05% was considered to be statistically significant.

3. Summation

3.1 Results

3.1.1 Patient Characteristics and Outcome

The treatment groups were well balanced with respect to baseline demographic and physiological characteristics (**Table 1**). Both groups had similar age, gender, mechanism of injury (70% MVA), injuries (67% head injury), ISS (23), pre-hospital time (2 hours) and pre-hospital fluid (2.5 liters). HS patients required significantly less fluid and blood during resuscitation, less mechanical ventilation, had shorter ICU stay and less infections. Importantly, there were no deaths in those patients treated with HSD, while two patients (14%) died after receiving NS resuscitation.

Table 1. Demographics and Baseline Characteristics

	Control (n=14)	HSD (n=10)	*p* value
Age, mean (SD), years	47.5 (15.9)	49.3 (16.7)	.759
Sex male, no. (%)	9 (64%)	7 (70%)	
ISS, mean (SD)	25.9 (10.3)	26.3 (11.4)	.83
Mechanism injury – MVA, no. (%)	9 (65%)	8 (80%)	
Fall, no. (%)	1 (7%)	2 (20%)	
Other, no. (%)	4 (28%)	0 (0%)	
Transferred from other institution, no. (%)	**5 (36%)**	**7 (70%)**	
Time - pre-hospital, mean (SD), min	110.5 (66.9)	172.4 (82.9)	.49
in the Trauma Room, mean (SD), min	114.6 (53.5)	164.5 (95.7)	.24
Lowest systolic BP Trauma Room, mean (SD)	90 (22.7)	80 (15.6)	.31
Highest HR Trauma Room, mean (SD)	114 (17.1)	110 (12.6)	.29
Crystalloid - **pre-hospital, mean (SD), ml**	**835 (855)**	**2144 (1343)**	**.048**
trauma Room, mean (SD), ml	4542 (2758)	3689 (1865)	.28
total first 24h, mean (SD), ml	8080 (2736)	7796 (3189)	.75
Blood - pre-hospital, mean (SD), units	0.5 (1.16)	1.22 (1.7)	.27
trauma Room, mean (SD), units	1.56	1.5	.62
total first 24h, mean (SD), units	4.36 (6.77)	2.2 (2.9)	.38
Colloids - **total first 24h, mean (SD), ml**	**696 (773)**	**361 (377)**	**.02**
LOS - total hospital stay, mean (SD)	**27.4 (11.7)**	**36.9 (43.7)**	**.048**
- ICU stay, mean (SD)	8 (8.2)	7.9 (6.8)	.3
Patients operated first 24h, no. (%)	10 (71.4%)	6 (60%)	
Number surgical procedures/patient, mean (SD	2.6 (2)	2.2 (2)	.21
Complications – vent time, mean (SD), days	5.3 (6.2)	4.3 (7.2)	.91
Pneumonia, number patients	1.43 (.51)	1.3 (.48)	.22
MOD score admission/highest			
Death	**2 (14.3)**	**0**	.25

3.1.2 Serum Sodium and Osmolality

Serum sodium levels were significantly higher in the HSD resuscitated patients at all time points after resuscitation (**Figure 3**). Serum osmolality was significantly increased in the HSD group at 6-h (**Figure 4**).

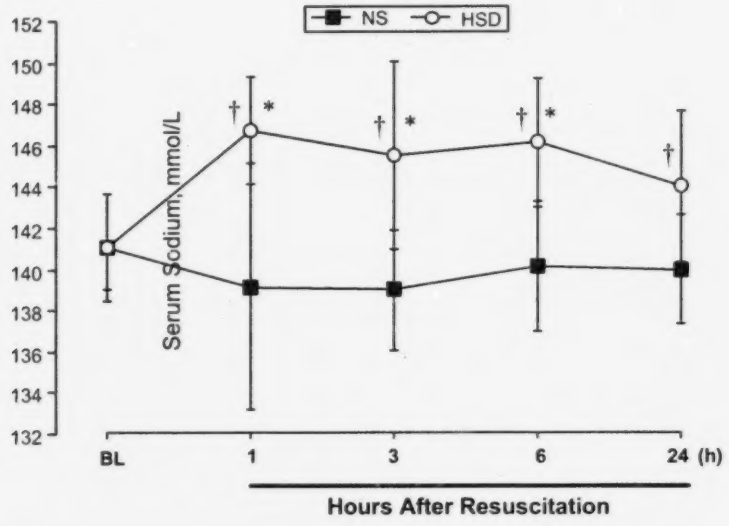

Figure 3. Serum Sodium Concentration.

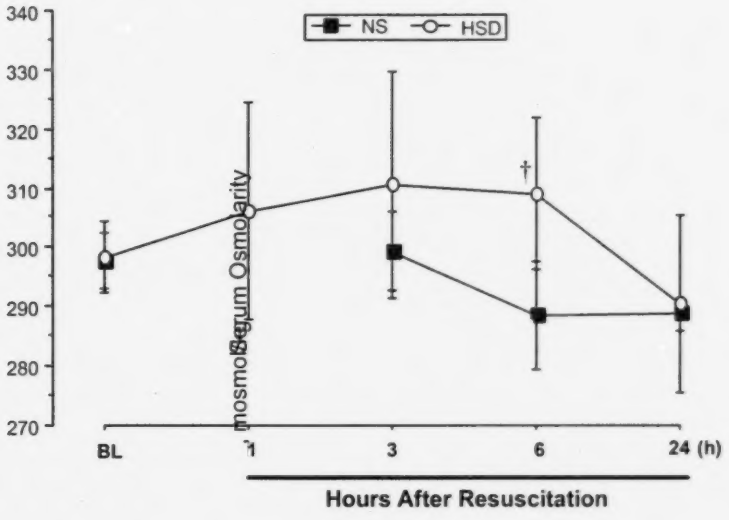

Figure 4. Serum Osmolality.

ranulocyte and Monocyte Counts and Percent Expression of CD11b and CD62L at Baseline and After Resuscitation According to Treatment Group

	Normal Saline (NS) (n = 10)				Hypertonic Saline (HS) (n = 10)				
ine	1h	3h	6h	24h	Baseline	1h	3h	6h	24h
3 6.9)	14.4 (10.1-18.8)	12.5 (9.1-15.9)*	10.9 (8.4-13.3)*	11.1 (8.7-13.1)*	13.1 (10.8-15.2)	12.5 (10.1-14.9)	12.3 (10.2-14.7)*	10.8 (9.3-12.2)*	9.6 (7.8-11.1)*
) 4.0)	12.2 (8.6-15.8)	10.1 (7.0-13.2)	8.8 (6.7-13.4)*	8.5 (6.3-10.4)*	10.9 (10.4-13.7)	10.6 (8.5-12.6)	10.3 (8.4-12.2)	9.1 (7.5-10.5)*	7.7 (6.0-9.4)*
3)9.6)	98.9 (98.1-99.8)	98.8 (97.8-99.7)	98.9 (97.8-99.8)	98.6 (97.6-99.8)	98.52 (97.6-99.3)	98.7 (98.4-99.5)	99.1 (98.7-99.3)	98.8 (98.0-99.6)	98.7 (98.3-99.2)
7)9.9)	96.1 (90.1-99.9)	97.9 (95.1-99.8)	99.0 (96.7-99.7)	97.1 (94.4-99.7)	96.3 (92.2-99.8)	96.9 (94.4-99.2)	96.8 (94.6-99.1)	97.8 (96.1-99.3)	96.3 (91.5-99.9)
3).82)	0.58 (0.29-0.86)	0.57 (0.34-0.79)	0.50 (0.32-0.67)	0.58 (0.25-0.89)	0.50 (0.35-0.62)	0.36 (0.24-0.45)	0.42 (0.27-0.55)	0.43 (0.33-0.51)	0.45 (0.29-0.58)
3)9.8)	97.3 (92.5-99.7)	96.9 (93.9-99.6)	96.8 (92.7-99.7)	97.0 (90.9-99.8)	96.1 (88.2-99.9)	97.3 (93.0-99.8)	96.1 (90.4-99.9)	95.5 (89.3-99.8)	96.6 (92.1-99.6)
7)9.8)	97.3 (93.1-99.8)	97.7 (94.2-99.6)	98.2 (95.8-99.5)	98.0 (95.3-99.6)	96.3 (89.2-99.9)	96.8 (90.6-99.8)	97.3 (92.4-99.7)	97.0 (92.5-99.6)	96.2 (92.1-99.8)

3.1.3 Peripheral Blood Leukocyte Counts

Table 2 shows the changes in circulating leukocyte subset concentrations in both NS and HSD resuscitated patient groups over the experimental time period. Baseline counts for total leukocytes, neutrophils, and monocytes were similar in both NS and HSD treatment groups. Resuscitation significantly ($P < .01$) reduced circulating leukocyte and granulocyte counts over time irrespective of the type of solution administered; whereas, monocyte counts remained unchanged in both groups throughout the experimental period.

3.1.4 Cellular Adhesion Molecule Expression

The proportion of circulating granulocytes and monocytes expressing CD11b and CD62L were not found to be significantly different between treatment groups or over time (Table 1). Changes in the surface expression (i.e., receptor density, measured as MFI) of CD11b and CD62L by unstimulated and LPS-stimulated blood neutrophils and monocytes were, however, significantly altered by the experimental treatment (**Figures 5 and 6**, respectively). In general, neutrophilic expression of the selected cellular adhesion molecules was affected more dramatically by the experimental treatment than was monocytic expression. Post-traumatic resuscitation of patients with NS provoked a biphasic upregulation of unstimulated neutrophilic CD11b expression (**Figure 5A**); levels rose 3-h after resuscitation (7%; $P < .005$), dropping slightly at 6-h, only to rise again after 24-h (9%; $P < .01$) as compared with baseline values. By contrast, HSD infusion led to a modest downregulation of CD11b surface expression 3-h (6%; $P < .05$) after resuscitation, which was sustained over the 24-h (7%; $P < .01$) sampling period. Furthermore, significant intergroup differences were observed over this time period, peaking 24-h post-resuscitation (12%; $P < .001$). On average, LPS-stimulation elicited a 16% increase ($P < .001$) in neutrophil CD11b surface expression relative to the unstimulated condition (**Figure 5B**), but an apparent trend toward lower CD11b expression in the HSD group was not statistically significant ($P = .105$).

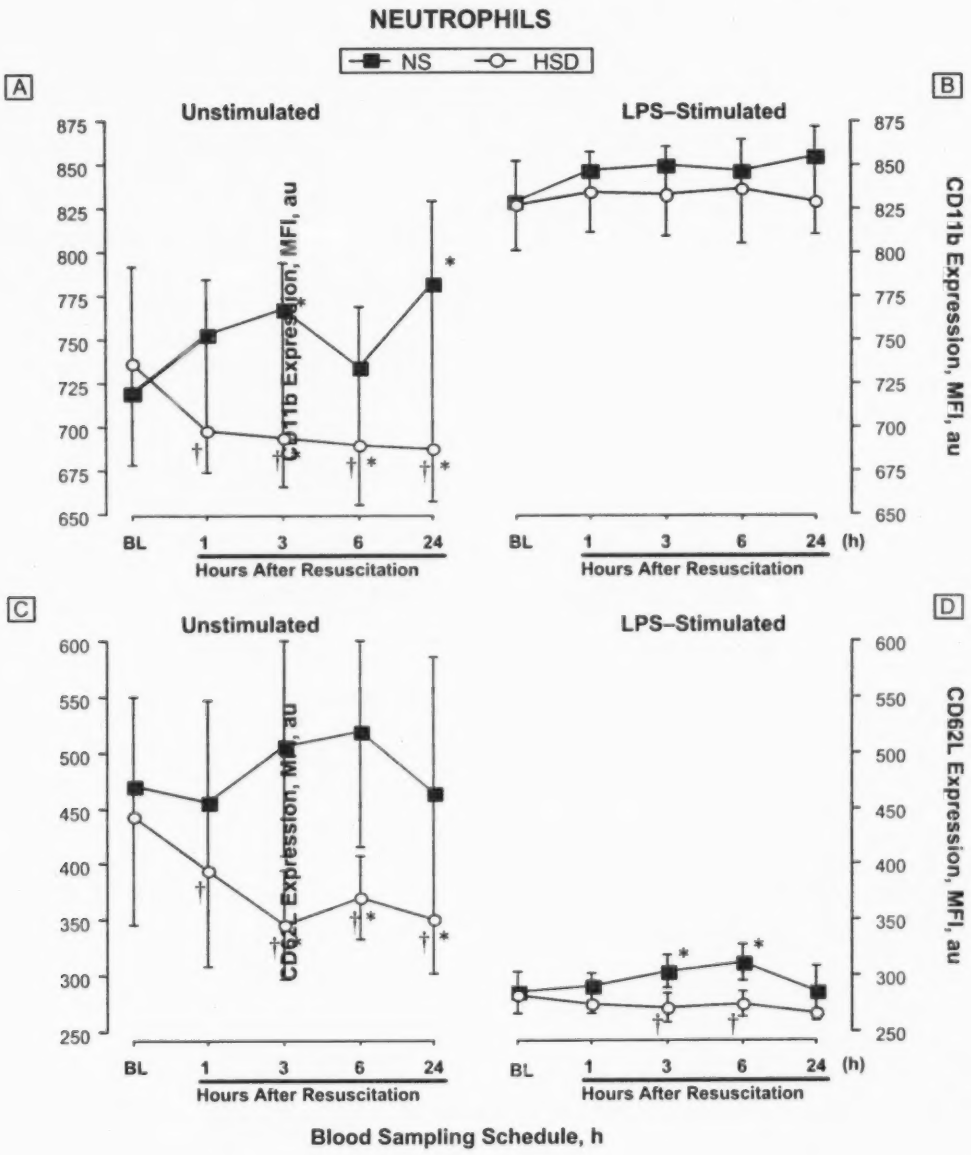

Figure 5. Neutrophil Adhesion Molecule Expression. Comparison of normal saline (NS) and hypertonic saline-dextran (HSD) treatment on serial changes in cell surface expression of CD11b and CD62L by unstimulated and LPS-stimulated whole blood neutrophils from trauma patients with hemorrhagic shock. Mean fluorescence intensity (MFI) values in arbitrary units (au) were used to compare the amounts of cell surface marker expression. The vertical error bars represent the 95% confidence intervals (CIs) of each mean value. Asterisk, significant difference compared to baseline (P <.05); dagger, significant difference between trials (P <.01).

In patients resuscitated with NS, unstimulated neutrophil CD62L surface expression did not change significantly over time (**Figure 5C**), despite a trend towards increased levels 6-h after resuscitation ($P = 0.07$). Nevertheless, HSD resuscitation resulted in a significant loss of CD62L expression (up to 22%; $P < .001$) between 3-h and 24-h after resuscitation. Moreover, intergroup comparisons demonstrated that CD62L surface expression was up to 30% ($P < .0001$) lower in patients receiving HSD as compared to the corresponding time points following NS resuscitation. In contrast to the LPS-stimulated upregulation of CD11b expression, neutrophil activation provoked a 26% ($P < .0001$) reduction in the average CD62L surface expression. In the NS group, LPS-stimulation led to enhanced expression of CD62L at 3-h ($P < .01$) and 6-h ($P < .001$) after infusion (**Figure 5D**). Although CD62L levels remained relatively stable over the sample period, significant ($P < .0001$) intergroup differences were observed at 3-h and 6-h following HSD infusion relative to NS resuscitation.

In comparison to the changes observed for neutrophil adhesion molecule expression, monocyte alterations were relatively modest (**Figure 6A–D**). Despite an apparent early rise in unstimulated CD11b expression by blood monocytes following resuscitation with NS, differences in CD11b were not statistically significant over time ($P = .946$) or between resuscitation treatment groups ($P = .221$). LPS-stimulation augmented average monocytic CD11b expression by 14% ($P < .0001$), whereas, resuscitation with HSD significantly blunted ($P < .05$) CD11b responsiveness to LPS after 3-h as compared to NS (**Figure 6B**). In patients receiving NS, unstimulated CD62L expression tended to rise 1-h after resuscitation, reaching statistical significance by 24-h ($P = .01$). By comparison, unstimulated CD62L expression remained unchanged following HSD resuscitation; however, once again, these levels were significantly lowered 3-h, 6-h (14%; $P < .02$) and 24-h (21%; $P < .001$) after resuscitation as compared to NS. Similar to neutrophilic CD62L expression, LPS-stimulation elicited a 25% ($P < .0001$) loss of CD62L surface expression as compared to unstimulated levels. However, no significant treatment effects were observed throughout the sampling period for LPS-stimulated CD62L expression.

As shown in **Figure 7**, circulating concentrations of sCD62L were unchanged 1-h following resuscitation in both NS and HSD-treated patients. By 6-h after infusion, patients receiving NS showed a 22% ($P < .05$) reduction in sCD62L concentration, by comparison HSD treated individuals exhibited a 25% increase ($P < .01$) in sCD62L levels at this time point, with respect to baseline values. These respective trends persisted 24-h after resuscitation. Comparison between NS and HSD infusion demonstrated significantly higher sCD62L concentrations at 3-h (30%; $P < .01$), 6-h (48%; $P < .001$) and 24-h (27%; $P < .01$) after resuscitation.

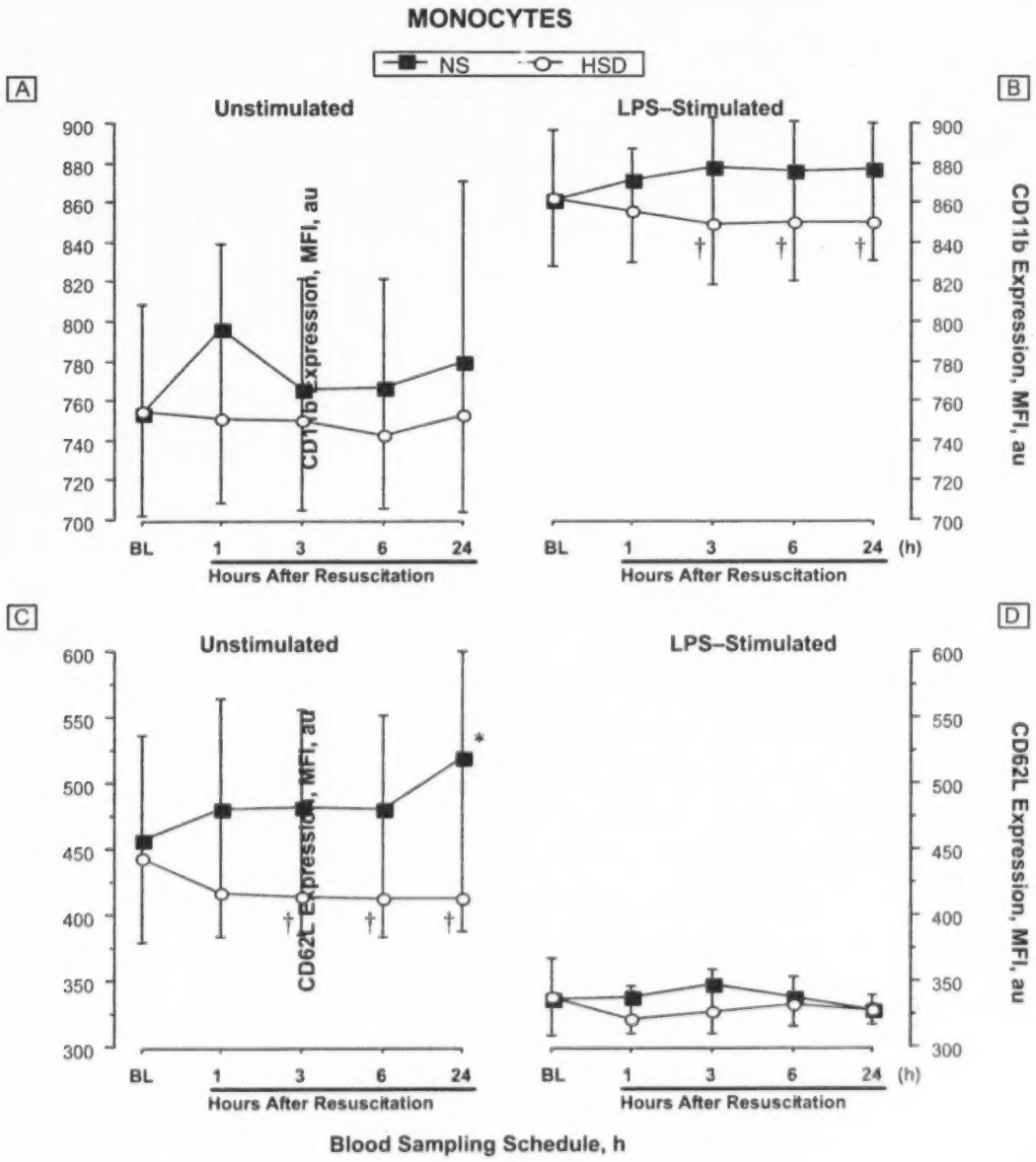

Figure 6. Monocyte Adhesion Molecule Expression. Comparison of normal saline (NS) and hypertonic saline-dextran (HSD) treatment on serial changes in cell surface expression of CD11b and CD62L by unstimulated and LPS-stimulated whole blood monocytes from trauma patients with hemorrhagic shock. Mean fluorescence intensity (MFI) values in arbitrary units (au) were used to compare the amounts of cell surface marker expression. The vertical error bars represent the 95% confidence intervals (CIs) of each mean value. Asterisk, significant difference compared to baseline (P <.05); dagger, significant difference between trials (P <.01).

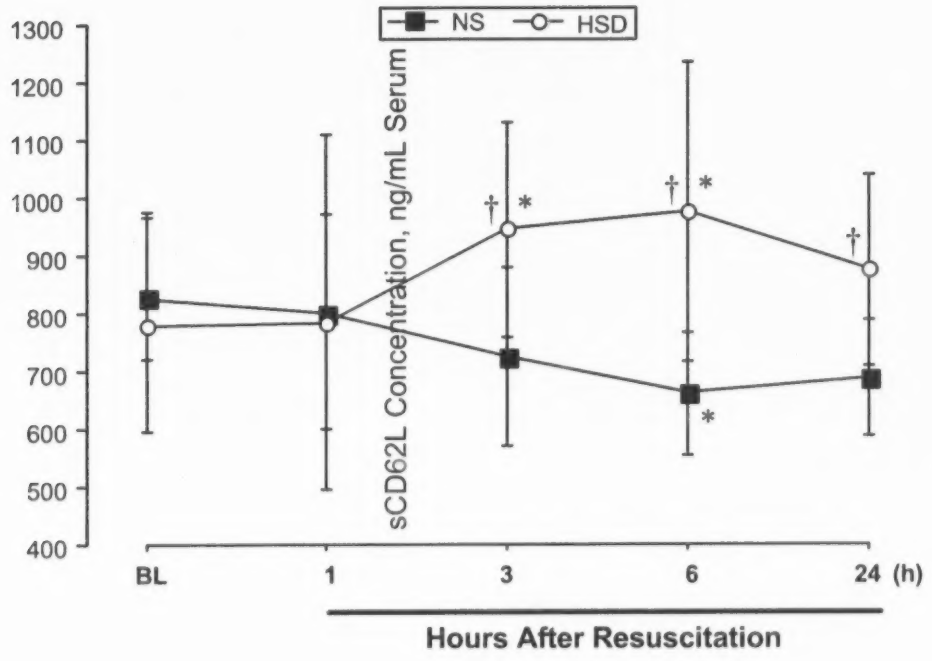

Figure 3. Soluble L-Selectin Concentration. Comparison of serial changes in sL-selectin concentrations (ng/mL) in the serum of patients treated with normal saline (NS) and hypertonic saline-dextran (HSD). The vertical error bars represent the 95% confidence intervals (CIs) of each mean value. Asterisk, significant difference compared to baseline ($P < 0.05$); dagger, significant difference between trials ($P < 0.01$).

3.2 Discussion

This is the first human trial examining the HS/D immunological effects in trauma victims. This study demonstrated the clinical efficacy and safety of applying a small-volume resuscitation strategy, using hypertonic saline/dextran (HS/D) in hypotensive hemorrhagic patients. The key findings are that HS/D patients required significantly less fluid and blood during resuscitation, less mechanical ventilation, had shorter ICU stay and suffered from less infections. Importantly, the use of HS/D did not adversely affect patient outcome and survival was equal.

Total leukocyte, neutrophil, monocyte counts were similar. HS/D resuscitation blunted neutrophil activation by abolishing shock-induced CD11b up-regulation and causing extensive CD62L shedding. These effects mirror animal studies indicating that HS/D has significant immunomodulatory activity and acts as an anti-inflammatory agent.

The finding of beneficial immunological effects is consistent with animal studies, providing "proof of principle" for larger clinical trials. Furthermore, HS/D may be an effective immunomodulatory agent in the broader range of surgical conditions caused by ischemia/reperfusion injury.

3.3 Conclusions

Hypertonic saline is a safe and effective alternative to normal saline, which can be used in smaller amounts to resuscitate injured soldiers. This has enormous logistical implications in transporting thousands of litres of fluid to the battlefield. A 250 mL bag of hypertonic saline is easily carried by corpsmen, can be infused within minutes and, because of its long-lasting effects, reduces the need for other fluids. Moreover, hypertonic saline also exerts beneficial anti-inflammatory effects, which can reduce post-traumatic multiple organ dysfunction. Thus, the adoption of a small-volume hypertonic fluid resuscitation strategy for treating combat casualties on the battlefield has the potential to re-define the CF operational doctrine and aid in the provision of state-of-the-art trauma care to forward-echelon medics where it is needed most. Hypertonic fluid resuscitation has the capacity to improve overall survival of combat casualties by increasing the number of casualties who survive delayed evacuation and secondary organ injury, while at the same time enhancing the operational capability of military medical personnel in austere combat environments, resulting in conservation of medical manpower, reduced reliance on field hospitals, and reduced acute and long-term military health care costs. More effective and efficient front-line operational medicine requires that the deployable CF medical support structure overcome logistical constraints to reduce its in-theatre medical footprint and lift requirements associated with forward positioning of medical care assets.

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14. ABSTRACT

(U) It is well established that severe trauma with hemorrhagic shock produces significant immunomoduation, including enhanced PMN activation, adherence and emigration into tissues, along with the induction of inflammatory cytokine cascades, which collectively contribute to the systemic inflammatory response syndrome (SIRS), frequently culminating in acute respiratory distress syndrome (ARDS) and multiple organ dysfunction syndrome (MODS). The hemodynamic and physiological benefits of hypertonic saline / dextran solutions (HS/D) have been evaluated extensively in animal and human research, and efficacy has been suggested for the treatment of hemorrhagic shock. Work from our research group and others has substantiated that in comparison to large volume crystalloid resuscitation, small volume resuscitation with HS/D has the remarkable capacity to reduce potentially deleterious immunologic complications. However, human clinical studies evaluating the cellular and molecular immune consequences of small-volume HSD resuscitation are lacking; also there is need to investigate modified fluid formulations incorporating novel antiinflammatory agents such as the antioxidant N-acetylcysteine.

(U) Il est bien établi qu'un traumatisme grave s'accompagnant d'un chox hémorragique produit un effet immunomodulateur important, notamment une activation accrue des PMN, l'adhérence et la migration dans les tissus ainsi que l'induction de cascades cytokiniques inflammatoires, qui contribuent collectivement au syndrome de réponse inflammatoire systémique (SRIS), aboutissant souvent au syndrome de détresse respiratoire aiguë (SDRA) et au syndrome de défaillance multiorganique (SDMO). Les bienfaits hémodynamiques et physiologiques des solutions hypertoniques salines/dextran (HSD) ont été évalués amplement dans des recherches chez l'animal et l'homme, et leur efficacité a été évoquée pour le traitement du choc hémorragique. Les travaux de notre groupe de recherche et d'autres équipes ont confirmé que, comparée à une réanimation au moyen d'un fort volume de cristalloïdes, la réanimation au moyen d'un faible volume de solution HSD a la remarquable faculté de réduire les complications immunologiques potentiellement délétères. Cependant, il faudrait des études cliniques évaluant chez l'homme les conséquences immunes cellulaires et moléculaires de la réanimation au moyen d'un faible volume de solution HSD; de plus, il faudrait étudier des formules liquides modifiées comportant des agents anti-inflammatoires nouveaux, tels que la N-acétylcystéine antioxydante.

15. KEYWORDS, DESCRIPTORS or IDENTIFIERS

(U) fluid resuscitation; clinical human trials; inflammatory response; immune function; cellular adhesion molecules